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Effects of Dietary Iron Overload on Glutathione Peroxidase Knockout Mice

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ABSTRACT

Excess iron (Fe) intake has been associated with an increased risk of cardiovascular disease in humans, presumably the result of increased oxidative stress. Previous work by us has shown that feeding a high-Fe diet to selenium (Se)-deficient weanling mice for 4 wk resulted in elevated plasma cholesterol and triglycerides and increased hepatic thiobarbituric acid reactive substances (TBARS). Here, we report the effect of Fe overload in mice lacking cellular glutathione peroxidase (GPX1 knockout [KO] mice), the selenoenzyme thought to account for much of the antioxidant action of Se. Four groups of 9–13 weanling wild-type (WT) or GPX1 KO mice were randomly assigned, then fed either an Fe-adequate (35 ppm Fe) or high-Fe (1100 ppm Fe) casein-based diet for 4 wk. Iron was added as ferric citrate. Both diets also contained 0.2 ppm Se added as sodium selenite. As expected, liver GPX1 activity was essentially absent in the KO mice. Another Se parameter measured (hepatic thioredoxin reductase activity) did not vary across groups. Although liver Fe was elevated in mice fed the high-Fe diet, liver TBARS was largely unaffected either by mouse genotype or diet fed. Moreover, plasma lipids were not elevated in the Fe-overloaded GPX1 KO mice. Thus, decreased GPX1 activity cannot account for the pro-oxidant hyperlipidemic effects observed earlier in mice fed the high-Fe Se-deficient diet. This suggests that impairment of Se functions other than GPX1 activity may be responsible for the elevated plasma lipids and hepatic TBARS seen in the Fe-overloaded Se-deficient mice.

Index Entries: Oxidative stress; selenium; antioxidants; cardiovascular disease.

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INTRODUCTION

Iron (Fe) is an essential nutrient required by nearly all organisms. It is involved in biochemical reactions central to metabolism. Redox cycling of Fe, however, results in the production of potentially damaging reactive oxygen species. An association between high-Fe stores and risk of myocardial infarction has been reported *(1).* Similarly, carriers of the Fe-loading human hemochromatosis gene have increased risk of acute myocardial infarction *(2).* Other studies, however, failed to demonstrate a relationship between elevated Fe status and coronary heart disease *(3,4).* Moreover, the National Academy of Sciences recently concluded that currently available data do not provide convincing support for an association between elevated body Fe stores and coronary heart disease, although evidence to exclude Fe definitively as a risk factor is insufficient *(5).* Thus, the relationship between high-Fe stores and coronary heart disease remains unclear.

Selenium (Se) functions as an essential trace element found in the selenocysteine-containing enzyme glutathione peroxidase (GPX1; glutathione H_2O_2 oxidoreductase, EC 1.11.1.9) *(6).* This enzyme plays an important role in host antioxidant defense. Employing reduced glutathione, GPX1 catalyzes the reduction of hydrogen peroxide and fatty acid hydroperoxides produced by the peroxidation of polyunsaturated fatty acids. Previous work by us has shown that feeding a high-Fe diet to Se-deficient mice for 4 wk resulted in elevated plasma lipids *(7).* To determine whether decreased GPX1 activity in itself can result in pro-oxidant hyperlipidemia, we fed mice genetically lacking cellular glutathione peroxidase a high-Fe diet.

MATERIALS AND METHODS

Mice

The GPX1 knockout (GPX1 KO) and wild-type (WT) mice were kindly provided by Dr. Y.-S. Ho, Wayne State University (Detroit, MI). GPX1 KO mice were generated from the 129/SVJ × C57BL/6 mice *(8).* Four groups of 9–13 weanling 3-wk-old WT or GPX1 KO mice were fed either a Fe-adequate (35 ppm Fe) or high-Fe (1100 ppm Fe) casein-based diet. At the end of a 4-wk feeding period and following an overnight fast, mice were anesthetized and blood was collected by heart puncture. Mice were then killed by cervical dislocation. Liver was frozen in liquid nitrogen and stored at –80°C until processed. Mice were given free access to respective diet and distilled deionized water at all times. Mice were housed four to five to a cage at the USDA/ARS Beltsville Small Animal Facility. Protocols used in these experiments were approved by the USDA Institutional Animal Care and Use Committee.

Diets

A casein-based Fe-adequate and high-Fe diet was purchased from Harlan Teklad (Madison, WI). The Fe-adequate and high-Fe diets were supplemented with 35 mg Fe/kg diet and 1100 mg Fe/kg diet as ferric citrate, respectively. Both diets also contained 0.2 mg Se/kg diet as sodium selenite. The iron concentration of the diets was confirmed by Fe analysis.

Thiobarbituric Acid-Reactive Substance Analysis

Lipid peroxidation in liver was measured by a thiobarbituric acid-reactive substance (TBARS) assay *(9)* modified for high-performance liquid chromatography (HPLC) *(10).*

Protein and Enzyme Analyses

The liver was homogenized in 9 volumes of 0.154 *M* KCl solution. To obtain a postmicrosomal supernatant, homogenates were centrifuged (2500 *g*) for 10 min to remove cellular debris and then ultracentrifuged (105,000 *g*) for 2 h. Postmicrosomal supernatant protein concentration was determined using the Pierce BCA protein assay kit (Pierce, Rockford, 1L) employing bovine serum albumin (BSA) as a standard. Enzyme activities for GPX1 and thioredoxin reductase were measured as previously described *(11,12).* Both assays were modified to work in a 96-well microplate format *(13).*

Hemoglobin Analysis

Blood was collected into heparinized syringes, and hemoglobin concentration was measured by the cyanmethemoglobin method (catalog no. 525A, Sigma).

Cholesterol and Triglyceride Analyses

Blood was collected into heparinized syringes and plasma was obtained upon centrifugation. Plasma triglyceride and plasma cholesterol were measured by an automated procedure employing an Élan Diagnostics Atach 8000 (Élan Diagnostics, Smithfield, RI) and triglyceride (catalog no. 589-008, Élan) and cholesterol (catalog no. 516-018, Élan) reagents.

Iron Analysis

Dietary Fe and liver Fe concentrations were determined employing a wet/dry-ashing procedure and flame atomic absorption spectrometry *(14).*

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) followed by Tukey's pairwise comparison when p was significant ($p < 0.05$). SigmaStat software (SPSS, Chicago, 1L) was used to perform all statistical analyses. Data in Figs. 1–3 are expressed as means ± SEM.

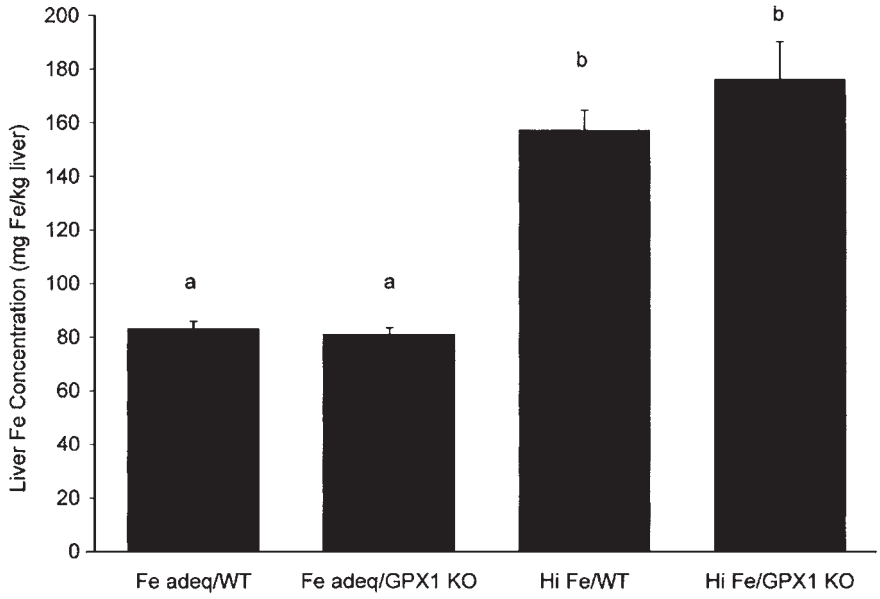

Fig. 1. Liver Fe concentration in WT and GPX1 KO mice fed Fe-adequate and high-Fe diets. Each bar represents the mean ± SEM (n = 9–13 mice). Bars not sharing a common letter are significantly different ($p < 0.05$). Abbreviations: WT, wild type; GPX1 KO, glutathione peroxidase knockout.

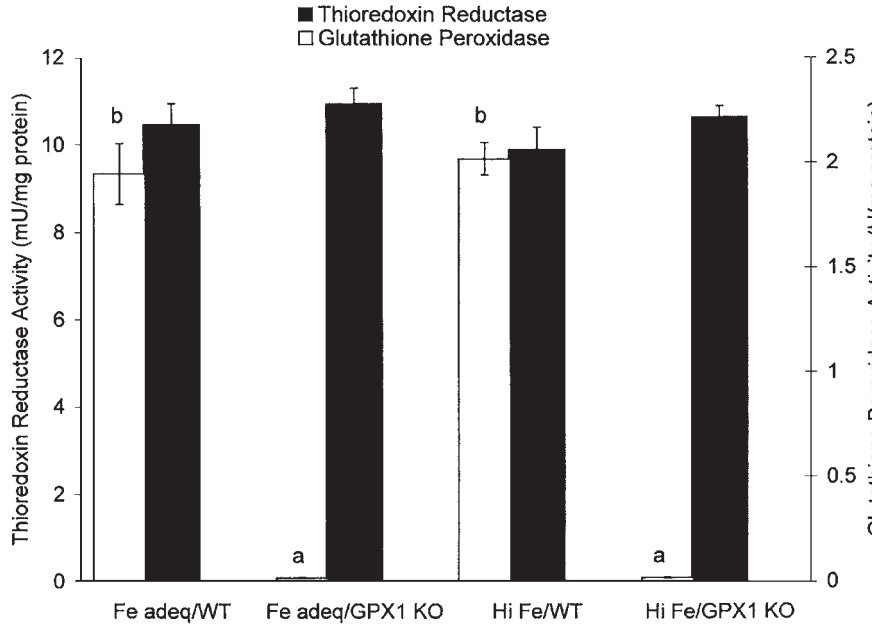

Fig. 2. Liver thioredoxin reductase activity and glutathione peroxidase activity in WT and GPX1 KO mice fed Fe-adequate and high-Fe diets. Each bar represents the mean ± SEM (n = 9–13 mice). Bars for glutathione peroxidase activity not sharing common letters are significantly different ($p < 0.05$). Abbreviations: WT, wild type; GPX1 KO, glutathione peroxidase knockout.

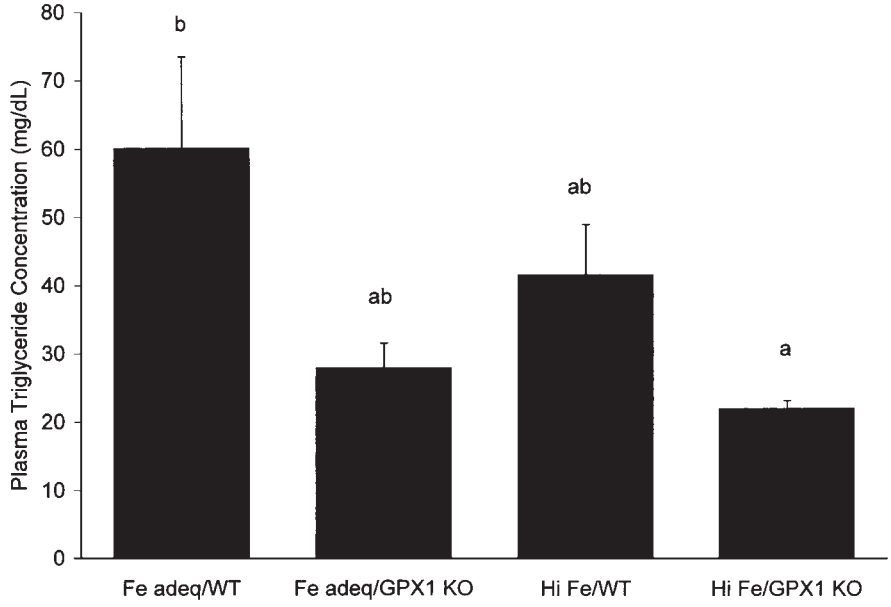

Fig. 3. Plasma triglyceride concentration in WT and GPX1 KO mice fed Fe-adequate and high-Fe diets. Each bar represents the mean ± SEM (n = 9–13 mice). Bars not sharing a common letter are significantly different ($p < 0.05$). Abbreviations: WT, wild type; GPX1 KO, glutathione peroxidase knockout.

RESULTS

As shown in Fig. 1, liver Fe stores were elevated in both GPX1 KO and WT mice fed the high-Fe diet. Mouse genotype, however, had no effect on liver Fe stores in either normal or Fe-overloaded mice. Hemoglobin concentration did not vary across groups (data not shown). As expected, liver GPX1 activity was nearly zero in mice lacking cellular glutathione peroxidase (GPX1 KO), whereas liver GPX1 activity was not affected by consumption of the high-Fe diet (Fig. 2). Liver thioredoxin reductase activity, however, did not vary across groups (Fig. 2). Mouse genotype and diet had no effect on liver TBARS (data not shown). Similarly, mouse genotype and diet had no effect on plasma cholesterol (data not shown). Plasma triglycerides, however, were decreased in GPX1 KO mice, resulting in lower plasma triglycerides in GPX1 KO mice fed the high-Fe diet compared to WT mice fed the Fe-adequate diet (Fig. 3).

DISCUSSION

In the present study, mice lacking cellular GPX1 activity fed high-Fe diets for 4 wk did not possess elevated plasma lipids or hepatic TBARS.

Previous work by us had shown that feeding mice a high-Fe Se-deficient diet for 4 wk resulted in elevated plasma cholesterol and triglycerides and increased hepatic TBARS *(7).* In our study, GPX1 activity in GPX1 KO mice was essentially absent, as shown previously in mice fed a Se-deficient diet. Thus, decreased GPX1 activity cannot account for the pro-oxidant hyperlipidemic effects observed earlier in mice fed the high-Fe Se-deficient diet.

Selenium functions as an essential trace element found in the selenocysteine-containing enzyme GPX1 *(6).* This enzyme is thought to account for much of the antioxidant action of Se. However, three additional Se-containing glutathione peroxidases exist in other cell fractions and tissues of the body that can metabolize hydrogen peroxide and lipid hydroperoxides *(15).* In addition, Se is contained in other selenoenzymes, including the deiodinases, responsible for regulating thyroid hormone *(16)* and thioredoxin reductase, involved in ribonucleotide reduction and redox control of certain enzymes, receptors, and transcription factors *(17).* Other Se-containing proteins include selenoprotein P *(18)* and selenoprotein W *(19)* whose functions are unclear. Thus, reduced Se status as a result of dietary Se deficiency would decrease GPX1 activity but would also affect other Se-containing enzymes as well.

Our data suggest that elimination of GPX1 genetically does not result in elevated plasma lipids and increased hepatic TBARS, as was shown previously in dietary Se deficiency in Fe-loaded mice. Thus, impairment of Se functions other than GPX1 activity may be responsible for the elevated plasma lipids and hepatic TBARS seen in the Fe-overloaded Se-deficient mice.

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